PATENT
ATTORNEY DOCKET NO. 051501-0305443

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Michael Croft, et al.
Serial No. : 10/661,358
Filed : 09/11/2003
Title : METHODS OF TREATING OX40 MEDIATED RECALL IMMUNE RESPONSES

Art Unit : 1644
Examiner : Ouspenski, Ilia

Assistant Commissioner for Patents
Washington, DC 20231

SUPPLEMENTAL DECLARATION UNDER 37 C.F.R. §1.131

Dear Sirs:

We, Dr. Michael Croft and Dr. Shahram Salek-Ardakani, do hereby declare and state that:

1. We are the inventors of the subject matter described and claimed in United States Patent Application Serial No. 10/661,358, filed September 11, 2003, entitled: "METHODS OF TREATING OX40 MEDIATED RECALL IMMUNE RESPONSES."

2. We are familiar with the prosecution history of Application Serial No. 10/661,358.

3. We understand that the Examiner has cited Arndt *et al.* (U.S. Patent Application Publication No. 2004/0009174 A1) under 35 U.S.C. §102(e) and §103(a) against claims 1 to 4, 6, 7, 11, 15 to 18, 23 to 31, 33 to 39 and 69 to 76 of Application Serial No. 10/661,358.

4. We submit that Arndt *et al.* (U.S. Patent Application Publication No. 2004/0009174 A1) is not available as prior art under either of 35 U.S.C. §§102 and 103.

5. We were diligent from the time that claims 1 to 4, 6, 7, 11, 15 to 18, 23 to 31, 33 to 39 and 69 to 76, as presented in the accompanying Response were conceived prior to

December 18, 2001, the filing date of the Arndt *et al.* priority application (Serial No. 60/341,453), and up until September 11, 2002, the filing of provisional patent application serial no. 60/410,534, to which this application claims priority.

6. The studies relied upon that establish conception and diligence described herein were performed in the United States.

7. Evidence of conception of claims 1 to 4, 6, 7, 11, 15 to 18, 23 to 31, 33 to 39 and 69 to 76 is supplied in the form of copies of four pages from our laboratory notebooks (Exhibit A, dates redacted), each of which is labeled A1-A4.

8. In particular, studies were of mice (BL/6) sensitized with 20 ug OVA/Alum to induce asthmatic lung inflammation, and subsequently treated or untreated with anti-OX40L antibody. In brief, three groups of four mice, Groups A, B and C, (page 1) were immunized with OVA (day 0). Following immunization, Group A control mice were administered 150 ug rat IgG for 3 days (days 24-27), Group B mice were administered 150 ug anti-OX40L antibody for 3 days (days 24-27), and Group C mice were administered 150 ug anti-OX40L antibody for 4 days (days 27-31) (A1). Mice were challenged via the airways in a recall immune response with OVA (5 mg/ml) delivered in aerosol for 30 minutes each day during days 28-31. Mice were sacrificed after the last challenge and airway measurements performed to ascertain lung inflammation.

9. Cytokines, namely IL-4, IFN, IL-5 and IgE, were measured in lung lavages (see, "OX40L blocking Asthma," A2 and A4). IL-4, which is typically increased in asthma, was reduced by an average of approximately 4-fold in the anti-OX40L antibody treated Groups B and C mice (Avg 0.1965 and 0.228), compared to control Group A mice (Avg 0.884). IgE levels, which also typically increase in asthma (A2 and A4) was also

reduced in anti-OX40L antibody treated Groups B and C mice (Avg 942), compared to control Group A mice (Avg 1229). These studies therefore indicate that anti-OX40L antibody reduces clinical indicia of asthma.

10. Neutrophil, eosinophil, monocyte and lymphocyte numbers were also measured in lung lavages (A3 and A4). Eosinophil and Lymphocyte numbers and percentages are typically increased in the asthmatic lung (page 13, lines 22-29 of the specification). Eosinophils were reduced in the lung of anti-OX40L antibody treated Groups B and C mice (Avg 14% and 20%; 1.11 x 10^4 and 1.66 x 10^4 cells) compared to control Group A mice (Avg 37% total; 5.16 x 10^4 cells). Lymphocytes were also reduced by an average of approximately 6-fold in the lung of anti-OX40L antibody treated Groups B and C mice (Avg 1.3% and 1.1% total cells; 0.11 x 10^4 and 0.09 x 10^4 cells) compared to control Group A mice (Avg 7.8% total cells; 0.64 x 10^4 cells). These studies therefore indicate that anti-OX40L antibody reduces eosinophil and lymphocyte infiltration of lung associated with asthma.

11. Evidence of diligence for claims 1 to 4, 6, 7, 11, 15 to 18, 23 to 31 and 69 to 76 from prior to December 18, 2001, up until the September 11, 2002 filing of provisional patent application serial no. 60/410,534, to which this application claims priority is supplied in the form of copies of pages from our laboratory notebooks, Exhibits B-G (dates redacted). Each of Exhibits B-G include one or more representative studies performed during a time period prior to December 18, 2001, and up to September 11, 2002: Exhibit B (pages B1-B5); Exhibit C (pages C1-C12); Exhibit D (pages D1-D7); Exhibit E (pages E1-E24); Exhibit F (pages F1-F16); and Exhibit G (pages G1-G17).

12. Certain studies required the animals to develop a recall immune response. For these studies, there is an about one-month time interval between immunization of the animals and subsequent studies performed after the animals established a recall (memory) immune response. Since this about one month time period was necessary for the animals to establish a recall response this time period is an interval required for performing the studies. Analyses of samples from each study typically took an additional 2-3 weeks. Subsequent studies were typically not initiated until the end of the analysis of the previous study.

13. Exhibit B includes a study of IgE production in serum and antigen specific T cell proliferation in spleen and lung of animals. In brief, mice were immunized with antigen (Ovalbumin; OVA) and left for approximately 1 month to allow memory Th2 cells to develop. After establishment of a recall response, mice were challenged with aerosolized OVA. Pages B1-B3 are studies of treatment with anti-OX40L (RM134L) on IgE resulting from the recall response. Control is non-immunized animals. Groups A and G are OVA-alum immunized and OVA challenged animals with control antibody treatment. Groups B and D are immunized and challenged animals treated with anti-OX40L just before recall response. Groups C and E are immunized and challenged animals treated with anti-OX40L at the time of recall response. Strong OVA-specific T cell proliferation was observed in spleen and lung cell cultures from OVA- immunized and OVA-challenged mice (OVA/alum) but not in unimmunized OVA-challenged mice (alum), reflecting the memory effector response and that a functional primary response did not result from exposure to airborne antigen alone. Pages B4-B5 are studies of OVA-specific T cell proliferation in the spleen or lung in animals treated with anti-OX40L during the recall response (OVA-alum-RM134L).

14. Exhibit C includes kinetic studies with anti-OX40L on the recall (memory) asthmatic response. In brief, mice were immunized with antigen (OVA) and left for approximately 1 month to allow memory Th2 cells to develop. After establishment of a recall response, mice were challenged with aerosolized OVA once a day on four consecutive days. Bronchoalveolar lavage fluid (BALF) was harvested before OVA challenge and on days 1, 2, 3, 4 after aerosol OVA challenge. Pages C1-C4 are studies of the effect of anti-OX40L (RM134L) on accumulation of eosinophils, neutrophils, monocytes and lymphocytes into BALF in OVA immunized animals. Page C4: series 1 is alum; series 2 is OVA-alum; and series 3 is OVA-alum-RM134L. Pages C5-C12 are FACS analyses of percentages and absolute numbers of OX40+CD4+ cells in spleen and lymph node (LN) of OVA immunized animals treated with anti-OX40L.

15. Exhibit D includes studies with anti-OX40L on secondary and tertiary recall asthmatic responses. These studies took place over about 3 months. Pages D1-D5 are a FACS analysis of cellular infiltrate into BALF, lung, lung draining lymph nodes and spleen in animals treated with anti-OX40L. Pages D6-D7 are assays of cytokines IL-4 and IL-5 levels in spleen and lung at one month and two months (secondary and tertiary responses, respectively) of animals treated with anti-OX40L.

16. Exhibit E includes adoptive transfer studies to show OX40 involvement in recall immune responses. In brief, OVA specific OX40-deficient CD4 T cells were produced by crossing OX40-knock out mice with OT-II TCR transgenic mice. Conditions for *in vitro* generation of memory Th2 cells were optimized, *in vitro* generated Th2 cells were transferred into naive recipients, and then these mice were challenged with aerosolized OVA. Pages E1-E24 are FACS analyses of cells in lung, lymph node and spleen to

measure cell division of transferred Th2 cells and infiltration of lung, lymph node and spleen in animals challenged with OVA.

17. Exhibit F includes additional studies using the adoptive transfer system described in paragraph 17 above. *In vitro* generated Th2 cells were labeled with CFSE (a dye) and adoptively transferred into naive mice, which were then challenged intranasally with OVA. Pages F1-F12 is a FACS analysis of OX40-expressing ($OX40^{+/+}$) Th2 cells after OVA challenge to measure cell division of all $OX40^{+/+}$ T cells, and accumulation in recipient animal lymph nodes and in lung. Pages F13-F16 illustrates total and differential counts of transferred OX40-deficient ($OX40^{-/-}$) Th2 cells recovered from BALF of recipient animals.

18. Exhibit G includes kinetic studies of effects of anti-OX40L on recall (memory) asthmatic response. Pages G1-G15 show studies of BALF, lung, lung draining lymph nodes and spleens harvested before OVA challenge and on days 1, 2, 3, 4, after aerosol OVA challenge and cell counts determined, and the effect of anti-OX40L at the time of inhalation of aerosolized OVA on memory T cell accumulation in lung draining lymph nodes and lung. Pages G16-G17 are studies of levels of IL-5 and IL-4 cytokines in BALF determined on days 1, 2, 3, 4 in control unimmunized and OVA-challenged (A, open diamond), OVA/alum immunized and OVA-challenged (B, closed circles), and OVA/alum immunized and OVA-challenged with anti-OX40L animals (C, open circles).

19. We declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the

United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 7.6.07

Michael Croft, Ph.D.

Date: 7.6.07

Shahram Salek-Ardakani, Ph.D.